961

Isolation of the Sterols of the White Potato[1,2]

By Joseph J. Schwartz and Monroe E. Wall

The sterols of the white potato (*Solanum tuberosum* L.) were investigated at this Laboratory as part of a comprehensive study of potato constituents. Although the steroidal alkaloid solanidine[3] and an unidentified steroidal glycoside[4] have been isolated from potatoes, and Lindemann[5] has reported 3.5% sterols in fat extracted from industrial potato starch, a study of the literature indicated that no information was available on the nature of the free sterols.

A large batch of dried, unpeeled Maine Katahdin potatoes (1950 crop) was extracted with boiling isopropyl alcohol. The unsaponifiable fraction was obtained in the usual manner. Digitonin precipitation yielded an amount of sterols equal to 0.002% of the weight of potatoes on a moisture-free basis. This is close to the value 0.003% calculated from the data of Lindemann.[5] Pyridine cleavage of the digitonides gave 6.7 g. of crude sterol fraction. Conversion to the dinitrobenzoate followed by chromatography on alumina and Florisil[6] removed non-steroidal impurities but accomplished no significant separation of the sterols themselves. Fractional crystallization of the dinitrobenzoates followed by chromatography on silica gel gave two fractions differing about 6° in melting point, of which the higher-melting, less soluble fraction was the minor constituent. Both fractions were hydrolyzed. The major, more soluble, fraction after numerous crystallizations gave β-sitosterol. The sterol was characterized by the melting points and rotations of the free sterol, acetate, benzoate and dinitrobenzoate, which agreed well with literature values. In addition, the infrared spectrum was essentially identical to that given by Dobriner, Katzenellenbogen and Jones.[7]

The minor, less soluble fraction, was identified as stigmasterol. The melting points and rotations of the free sterol and the benzoate were in close agreement with literature values. Further, the infrared spectrum was identical to that of authentic stigmasterol.

Experimental[8]

Isolation of Crude Sterols.—Dried potatoes (276 kg.) containing 8.8% moisture were extracted in 5 batches with a total of 178 gal. of boiling 99% isopropyl alcohol. The residue from the alcohol was saponified with boiling 10% KOH–methanol solution. The mixture was treated in the usual manner, and 46.0 g. of unsaponifiable matter was obtained.

The sterols were isolated by a modification of method F of Sperry.[9] The unsaponifiables, dissolved in 1060 ml. of boiling absolute ethanol, were treated with 21.1 g. of digitonin (Merck) in 716 ml. of 80% ethanol. The mixture was boiled for one minute, 265 ml. of water was added, and the mixture was brought to a boil again. After standing overnight the digitonides were filtered off and washed. They were then treated with pyridine according to the method of Schoenheimer and Dam[10] to obtain the free sterols.

Preparation and Purification of Dinitrobenzoates.—The sterols (6.7 g.) were heated two hours on a steam-bath with an equal weight of 3,5-dinitrobenzoyl chloride and 35 ml. of pyridine. The mixture was treated with 1 ml. of water to decompose excess reagent and cooled. Extraction with ether in the usual manner yielded 9.5 g. of solids.

After chromatography on alumina and Florisil, with little improvement in melting point, the dinitrobenzoates were fractionally crystallized from ethyl acetate, and each fraction was chromatographed on a 1:1 mixture of silica gel–Hyflo Supercel.[6] Finally all the fractions were combined into two main fractions A and B. Fraction A, m.p. 204–215°, was less soluble than the lower melting fraction B, m.p. 202–207°.

Saponification and Purification of Sterols.—Fractions A and B were saponified with 5% KOH in methanol, extracted with ether in the usual manner, and crystallized from 95% ethanol. Each fraction was then digested with two small portions of warm light petroleum ether,[11] which removed waxy, non-steroidal impurities. The insoluble residues (A and B) were crystallized six times from 90% ethanol. Fraction B yielded β-sitosterol, 0.67 g., plates, m.p. 138–138.5°, $[\alpha]_D$ −35° (lit.[12] gives m.p. 136–137°, $[\alpha]_D$ −36.6°).

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 84.14; H, 12.36.

β-Sitosteryl Acetate.—The product was prepared from β-sitosterol in the usual manner by heating with acetic anhydride–pyridine for 1 hour at 90; plates from methanol, m.p. 127–129°, $[\alpha]_D$ −45°. (Lit.[12] gives m.p. 125–126°, $[\alpha]_D$ −41.0°.)

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 80.93; H, 11.47.

β-Sitosteryl Benzoate: rectangular plates from acetone,

(1) Article not copyrighted.

(2) This is paper XXIX in a series on steroids and steroidal sapogenins. Paper XXVIII, M. E. Wall, H. E. Kenney and E. S. Rothman, This Journal, **77**, Nov. 5 (1955).

(3) G. R. Clemo, *et al.*, *J. Chem. Soc.*, 1299 (1936).

(4) W. Völksen, *Arch. Pharm.*, **283**, 203 (1950).

(5) E. Lindemann, *Die Stärke*, **3**, 141 (1951).

(6) The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

(7) K. Dobriner, *et al.*, "Infrared Absorption Spectra of Steroids, an Atlas," Interscience Publishers, Inc., New York, N. Y., 1953, chart 58.

(8) Melting points were obtained with a Kofler hot-stage. Optical rotations were taken in chloroform at 25°.

(9) W. M. Sperry, *J. Biol. Chem.*, **113**, 377 (1937).

(10) R. Schoenheimer and H. Dam, *Z. physiol. Chem.*, **215**, 59 (1933).

(11) G. Soliman and W. Saleh, *J. Chem. Soc.*, 1506 (1954).

(12) E. S. Wallis and P. N. Chakravorty, *J. Org. Chem.*, **2**, 335 (1937).

m.p. 147–148°, $[\alpha]_D$ −13.3°. (Lit.[12] gives 146–147°, $[\alpha]_D$ −13.8°.)

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49. Found: C, 83.41; H, 10.43.

β-Sitosteryl 3,5-Dinitrobenzoate: Pale yellow plates from benzene–acetone, m.p. 211–213°, $[\alpha]_D$ −12.4°. (Lit.[12] gives 202–203°, $[\alpha]_D$ −10.4°.)

Anal. Calcd. for $C_{36}H_{52}O_6N_2$: C, 71.02; H, 8.61. Found: C, 71.28; H, 8.70.

Stigmasterol.—Fraction A, after two crystallizations from 95% acetone, was converted to the benzoate with pyridine–benzoyl chloride in the usual manner. After crystallization of the benzoate from acetone–benzene, the product was saponified. The free sterol, 0.05 g., was obtained as plates from 95% ethanol; m.p. 166–168°, $[\alpha]_D$ −46°. (Lit.[13] gives m.p. 168–169°, $[\alpha]_D$ −47.3°.)

(13) A. C. Ott and C. D. Ball, THIS JOURNAL, **66**, 489 (1944).

Stigmasteryl Benzoate.—Plates from benzene–acetone, m.p. 158–161°, $[\alpha]_D$ −25°. (Lit.[13] gives m.p. 160.5–161.5°, $[\alpha]_D$ −24.5°.)

Acknowledgments.—The authors wish to thank the Engineering and Development Section of this Laboratory for supplying the dried potatoes, J. W. Garvin for extracting them, and T. Perlstein for obtaining the unsaponifiable extract. Carbon–hydrogen analyses were made by Kathryn M. Zbinden under the supervision of C. L. Ogg. The infrared spectra were obtained by C. S. Fenske under the supervision of C. R. Eddy.